ANTIEPILEPTIC EFFECTS OF A COMBINATION OF SODIUM VALPROATE AND THE CALCIUM ANTAGONIST RYODIPINE ON A MODEL OF GENERALIZED METRAZOL-INDUCED EPILEPTIC ACTIVITY

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KEY WORDS: sodium valproate; calcium antagonist; ryodipine; metrazol; generalized epileptic activity; combination pathogenetic therapy.

Epileptization of neurons and generalization of epileptic activity (EpA) are effected by a complex series of extracellular, membrane, and intracellular mechanisms. The use of a particular anticonvulsant in the form of monotherapy in order to suppress an epileptic process is therefore by no means always effective. Considerations such as these were the grounds for an investigation of the principle of combination pathogenetic therapy (CPT) [4], namely the combined administration of substances acting on different components of the pathological process. Therapy of this kind, as experimental [5, 6] and clinical [3] studies have shown, gives a more complete effect and can result in administration of drugs in smaller doses.

A very important role in the mechanisms of epileptogenesis is played by disturbance of GABA-ergic inhibition [14] and an increased Ca^{2+} inflow into the neuron [1]. The aim of the present investigation was accordingly to study the efficacy of a combination of preparations acting on the above-mentioned mechanisms of epileptogenesis: sodium valproate, which potentiates GABA-ergic processes [12], and the calcium antagonist ryodipine (a new drug belonging to the 1,4-dihydropyridine class, synthesized at the Institute of Organic Chemistry, Academy of Sciences of Latvia). We showed previously, on models of focal and generalized EpA, that ryodipine possesses a marked antiepileptic action [2].

EXPERIMENTAL METHOD

Experiments were carried out on 114 male Wistar rats weighing 200-240 g. The animals were kept under ordinary animal house conditions and on a standard diet. Generalized EpA was induced by intraperitoneal injection of metrazol in a dose of 80 mg/kg. The effects observed were recorded visually for 60 min. Latent periods of the first seizures, and of the clonic and tonico-clonic phases (the animal falling on to its side) of the generalized convulsive reactions, and their duration, the severity of the convulsive reaction, duration of survival, and mortality, were determined. The severity of the convulsive reaction was assessed in points: 1) clonic convulsions, 2) repetitive clonic convulsions, 3) tonico-clonic convulsions with the animal falling on to its side, and 4) repetitive tonico-tonic convulsions and/or death of the animal. Considering the possible effects of various factors, the effects of valproate and ryodipine singly and in combination were studied in parallel tests at the same time as the effects of metrazol alone was tested. Drugs were injected intraperitoneally. There were two series of experiments. In the experiments of series 1 the drugs were used in doses at which each had a distinct antiepileptic effect: ryodipine (in a 30% solution of dimethyl sulfoxide) in a dose of 1 mg/kg 30 min before injection of metrazol, sodium valproate in a dose of 80 mg/kg

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TABLE 1. Effect of Sodium Valproate (80 mg/kg), Ryodipine (1 mg/kg), and a Combination of Both on Generalized EpA (Series 1, M ± m)

Group and number (n) of animals	LP1, sec	Clonic phase		Tonico-clonic phase		Severity of convulsions, points	Time of animal's death, sec	Number of animals dying
		LP2, sec	duration, sec	LP3, sec	duration of tonic phase, sec			
Control, Metrazol (n = 18)	49,7±2,4	58,2±2,5	25,6±1,9	242,8±45,9	22,7±1,5	3,61±0,1	1200,1±230,3	9
Valproate + metrazol (n = 8)	60,0±3,4	68,6±2,7 $p<0,01$	20,4±1,9	710,0±0,00	23,0±0,0	1,88±0,2 $p<0,001$	—	0 $p<0,025$
Ryodipine + metrazol (n = 12)	65,6±2,9 $p<0,001$	85,8±3,2 $p<0,001$	26,9±2,6	387,2±96,0	23,3±1,8	2,75±0,1 $p<0,001$	1320,0±0,00	1 $p<0,025$
Valproate + ryodipine + metrazol (n = 13)	76,3±4,8 $p<0,001$	133±18,6 $p<0,001$ $*p<0,05$ $**p<0,02$	18,7±3,2	—	—	2,08±0,1 $p<0,001$	—	0 $p<0,025$

Legend. Here and in Table 2: LP1, LP2, and LP3) Latent period of first seizure manifestations, of clonic and tonico-clonic phases of generalized convulsive reaction; p) compared with corresponding parameter in group of control animals: *p and **p) compared with corresponding value in group of animals receiving valproate or ryodipine respectively.

TABLE 2. Effect of Sodium Valproate (70 mg/kg), Ryodipine (0.75 mg/kg), and a Combination of Both on Generalized EpA (Series 2, M ± m)

Group and number (n) of animals	LP1, sec	Clonic phase		Tonico-clonic phase		Severity of convulsions, points	Time of animals' death, sec	Number of animals dying
		LP2, sec	duration, sec	LP3, sec	duration of tonic phase, sec			
Control, metrazol (n = 15)	37,4±1,5	48,5±2,9	22,0±2,5	126,0±8,5	19,7±2,7	2,73±0,2	421,2±125,0	6
Valproate + metrazol (n = 17)	33,7±2,4	47,9±3,9	17,5±1,3	475,0±156,9 $p<0,05$	17,5±9,4	1,88±0,2 $p<0,001$	675,0±119,2	2
Ryodipine + metrazol (n = 14								
Valproate + ryodipine + metrazol (n = 17)	35,0±2,6 45,2±2,6 $p<0,02$	44,8±2,6 96,4±8,6 $p<0,001$	21,6±2,0 24,0±2,5	246,8±68,2 —	16,2±1,2 —	2,78±0,2 1,29±0,1 $p<0,001$ $p<0,05$	408,0±75,0 —	6 0 $p<0,025$

10 min before injection of metrazol. In the experiments of series 2 the same drugs were used in smaller doses, which had a less significant antiepileptic effect: ryodipine 0.75 mg/kg, valproate 70 mg/kg. Control animals received the solvent only under identical experimental conditions and in the same volume (0.2 ml): a 30% solution of dimethyl sulfoxide or physiological saline.

The experimental results were analyzed by parametric and nonparametric statistical tests: Student's and Fisher's.

EXPERIMENTAL RESULTS

Injection of physiological saline and a 30% solution of dimethyl sulfoxide in the control experiments had no effect on the generalized convulsive reaction of the animals. Later these animals were considered together as a single control group for each series.

In the experiments of series 1 injection of metrazol caused tonico-clonic convulsions in the majority of the animals (in 17 of 18), with the animal falling on to its side (Table 1). Valproate in a dose of 80 mg/kg had a marked antiepileptic effect, by delaying development of the clonic phase of the generalized convulsive reaction, reduction of the severity of the convulsions, and reducing the number of animals with clonico-tonic convulsions. Not a single animal in this group died. Injection of ryodipine in a dose of 1 mg/kg also had a distinct antiepileptic effect in the form of lengthening of the latent period of the first seizure manifestations, delay of development of the clonic phase

of the generalized convulsive reaction, a decrease in the number of animals with tonico-clonic convulsions, and a decrease in the severity of the convulsions and their mortality. In response to a combination of valproate and ryodipine in doses of 80 and 1 mg/kg respectively, more marked suppression of EpA was observed than when these drugs were used separately in the same doses, in the form of delay of the clonic phase of the convulsions and the absence of a tonico-clonic phase in the animals; with respect to other parameters the antiepileptic effect was similar to that of the drugs when given separately (Table 1).

In the experiments of series 2 injection of metrazol caused a generalized convulsive reaction in seven of 15 control animals (Table 2). In the other animals (eight of 15) repetitive tonic convulsions occurred. Valproate in a dose of 70 mg/kg had an inhibitory effect, expressed as delay of development of tonico-clonic convulsions, a decrease in the number of animals with these convulsions, and a decrease in the severity of the convulsions. Ryodipine in a dose of 0.75 mg/kg had no significant effect on generalized EpA, with only a tendency for the latent period of the tonico-clonic convulsions to increase. In response to a combination of valproate and ryodipine in doses of 70 and 0.75 mg/kg respectively marked inhibition of generalized EpA was observed, in the form of an increase in the latent period of the first seizure manifestations, delay of development of the clonic phase of the generalized convulsive reaction, and a decrease in severity of the seizures (Table 2). Not a single animal in this group developed tonico-clonic convulsions and not a single animal died.

The results of this investigation indicate that a combination of valproate and ryodipine, in reduced doses (70 and 0.75 mg/kg respectively) gives a stronger antiepileptic effect than each of the two preparations given separately. The weaker potentiating effect of a combination of these two drugs in larger doses (80 and 1 mg/kg respectively) may be connected with the fact that under these conditions each drug itself had a marked antiepileptic action.

The mechanism of action of valproic acid and its salts is linked with their specific action on GABA metabolism, for valproate interacts with the enzyme systems responsible both for activation of synthesis and for degradation and inactivation of GABA [12]. Valproate thus potentiates GABA-ergic mechanisms which are realized through an increase in Cl^--conductivity [10, 15] and postsynaptic inhibition [7, 13]. The antiepileptic action of ryodipine, as a representative of the 1,4-dihydropyridine group, is associated with blockade of the Ca-inward current [1]. Combined administration of these substances, which act on different initial mechanisms of epileptogenesis, gives a potentiating effect. There is evidence that the use of nifedipine (1,4-dihydropyridine) with valproate or phenobarbital also gives a stronger antiepileptic effect against metrazol seizures in mice [9]. The effect of phenobarbital also is known to be realized through potentiation of GABA-ergic inhibition [11].

Elucidation of the problem of CPT of epilepsy and, in particular, the choice of combinations of antiepileptic agents, requires special study. The specific features of the action of anticonvulsants and the basic initial pathogenetic mechanisms of each form of experimental and clinical epilepsy must be taken into account. There is some evidence [9] that a combination of verapamil and diltiazem with valproate or phenobarbital does not potentiate the antiepileptic effect. Some Ca^{2+}-antagonists have a potentiating action on models of metrazol- and electroshock-induced seizures, but not on aminophylline-induced seizures in mice [8].

The investigations thus demonstrate the benefit of CPT in the form of a combination of valproate and the Ca^{2+} antagonist ryodipine, which are antiepileptic agents corresponding to the basic pathogenetic mechanisms of this form of epilepsy. The use of CPT is also indicated because, as previous investigations [3, 5, 6] showed, it can reduce the risk of side effects of each individual drug on account of the reduction of their dosage, a particularly important aspect in the case of prolonged treatment.

REFERENCES

1. R. N. Glebov and M. N. Karpova, Patol. Fiziol., No. 3, 57 (1990).
2. M. N. Karpova, O. Yu. Pankov, R. N. Glebov, et al., Byull. Éksp. Biol. Med., No. 11, 553 (1989).
3. V. M. Kovalenko, G. N. Kryzhanovskii, V. S. Kovalenko, et al., Zh. Nevropatol. Psikhiat., No. 6, 892 (1984).
4. G. N. Kryzhanovskii, Determinant Structures and Pathology of the Nervous System [in Russian], Moscow (1980).
5. G. N. Kryzhanovskii and A. A. Shandra, Byull. Éksp. Biol. Med., No. 11, 545 (1985).
6. G. N. Kryzhanovskii and A. A. Shandra, Farmakol. Toksikol., No. 4, 21 (1985).

7. F. Baldino and H. M. Geller, J. Pharmacol. Exp. Ther., **217**, 445 (1981).
8. S. J. Czuczwar, A. Chodkowska, Z. Kleinrok, et al., Eur. J. Pharmacol., **176**, 75 (1990).
9. S. J. Czuczwar, U. Malak, and Z. Kleinrok, Neuropharmacology, **29**, 943 (1990).
10. J. P. Gent and N. I. Phillips, Brain Res., **197**, 275 (1980).
11. I. Jurna, Antiepileptic Drugs, H. J. Frey and D. Janz (ed.), Berlin (1985), pp. 611-658.
12. W. Loscher, Antiepileptic Drugs, H. J. Frey and D. Janz (ed.), Berlin (1985), pp. 507-536.
13. R. L. MacDonald and G. K. Bergey, Brain Res., **170**, 558 (1979).
14. B. S. Meldrum, Antiepileptic Drugs, H. J. Frey and D. Janz (ed.), Berlin (1985), pp. 153-185.
15. M. K. Ticku and W. S. Davis, Brain Res., **223**, 218 (1981).